

Bronchial provocation with cat allergen: correlation between the individual IgE-CRIE pattern and the occurrence of a late allergic reaction

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Summary

Twenty-one mild asthmatic patients allergic to cat dander underwent a bronchial provocation test (BPT) with a cat extract. An early allergic response (EAR) was observed in all 21 patients and a late allergic response (LAR) in 8/21 patients. In the EAR, the patients with subsequent LAR had a greater fall in FEV₁. Their baseline FEV₁, and total dose of inhaled allergen were not significantly different from patients who did not develop a LAR, but their serum specific IgE level was higher. Crossed immunoelectrophoresis (CIE) of the same cat extract showed that it contained eight different proteins. An IgE-CRIE was obtained from all 21 patients, using radiolabelled anti-IgE and autoradiography. Radiolabelled standards allowed a semiquantitative scoring of the radiostaining. The CRIE pattern of the eight patients with LAR showed a higher score of radiostaining and a greater number of proteins bound to IgE. The two major allergen cat albumin and *Fel d I* bound equally to IgE of patients with and without LAR whereas another protein (antigen No. 7) bound to IgE of 100% of patients with LAR but of only 38% of patients without LAR. These data suggest that the pattern of the IgE response to specific proteins of a cat extract may be related to the occurrence of LAR after BPT with this allergen.

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Introduction

Bronchial provocation tests (BPT) are extensively used to study the mechanisms involved in asthma. Improved standardization of these tests has produced better reliability and safety, and has allowed the comparison of results obtained by different groups [1,2].

In BPT with allergen, two kinds of response have been observed. The early allergic response (EAR) occurs minutes after inhalation of the allergen and is defined as a drop in FEV₁ of at least 20%. EAR usually resolves spontaneously within 1–3 h. About half of the subjects have a second drop in FEV₁. This late allergic reaction (LAR) occurs after about 4–6 h, has a longer duration than EAR, and can last from hours to days, LAR is

poorly reversed by β -2-agonist drugs and is considered to be a better model of clinical asthma than EAR [3]. It is not known why some subjects react with an EAR only, while others have an EAR followed by a LAR.

Late allergic reactions have been described more than a century ago by Blackley [4] but their clinical importance was first emphasized by Herzheimer in 1952 [5]. Pepys and Booij-Noord *et al.* were among the first investigators to study the pathogenesis of the response to inhaled allergens [6,7]. One hypothesis was that LAR was due to an Arthus reaction but Dolovich *et al.* have demonstrated in the skin that LAR to allergen is an IgE-dependent mechanism [8]. Almost all inhaled allergens have been shown to be able to induce LAR. However, patients allergic to many allergens may develop LAR after exposure to some of these allergens and not to others [9]. The exact prevalence of LAR is difficult to establish. One reason is that the patients selected for BPTs may not

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reflect the whole asthmatic population since severe asthmatics are excluded from BPT studies for evident ethical reasons. Furthermore, non-specific and poorly understood co-factors, such as viral infections may play roles in the onset of allergic diseases [10]. Many aspects of the pathogenesis of LAR remain obscure even though extensive research has implicated many mediators and cells in this reaction [11,12].

Crossed immunoelectrophoresis (CIE) detects the different components of an allergen extract and is commonly used to isolate the proteins of such extracts [13]. CIE has been used extensively to compare the protein contents of different allergen extracts. Crossed radioimmuno-electrophoresis (CRIE) can be used to determine which of these extract components bind IgE of sensitized patients, and so determine the allergenic content of allergen extracts [14]. Analysis of cat dander extracts reveal at least eight proteins that bind IgE [15–17]. *Fel d1* and cat albumin are the main components of the allergenic activity of cat dander extracts [18–20].

The aim of this study was to determine whether subjects who react with a dual response to allergen BPT (EAR and LAR) have different IgE-responses to the individual allergenic proteins of a cat dander extract as measured by the CRIE method.

Materials and methods

Patients

Twenty-one asthmatic patients, 12 males, 9 females, age 18–43, mean age = 29, underwent a BPT with a cat extract (Pharmalgen® cat, Pharmacia, Uppsala, Sweden). These patients had mild asthma since they had never been hospitalized for severe asthma, and they did not need regular anti-asthmatic treatment. Allergy to cat was documented by a positive history and both, a positive skin-prick test (Pharmalgen® extract, 100 000 BU, Pharmacia, Uppsala, Sweden) and a positive RAST (class 2–4, Phadezim®, Pharmacia). All patients had experienced wheezing in the presence of cats but only one still had a cat at home. Ten patients also had a positive history and positive skin tests to house dust mites. Seven patients were also sensitized to pollens and three to dog dander. The patients had not suffered from any respiratory tract infections during the last 2 months, had not taken steroids for 3 weeks, no cromolyn and H1 antihistamines for 2 weeks, no non-steroidal anti-inflammatory drugs for 3 days, no theophylline for 2 days and no β -2-agonist drugs for 12 hr. All the patients were volunteers and the design of the study had been previously agreed by the ethical committee of our hospital.

Bronchial provocation test

The BPTs were performed in winter since some patients had positive skin-prick tests to pollens. The BPTs were started at 08.00 h and performed only if the baseline FEV₁ was greater than 70% of the predicted value. The reference FEV₁ was the best of three obtained on a Godart® spirometer (Statham, The Netherlands) after inhalation at tidal volume for 4 min of 1 ml of diluent (human albumin 0.3 mg, phenol 4 mg, NaCl 9 mg) delivered by a Novair® nebulizer (Carbamed, Switzerland).

The patients then inhaled the allergen extract in the same fashion. They inhaled increasing concentrations (100, 320, 1000, 3200, 10 000, 32 000, 100 000 BU) at 15 min intervals. Ten minutes after each allergen inhalation, the FEV₁ was measured. The inhalation dose progression was stopped at the allergen concentration which induced a FEV₁ drop $\geq 20\%$ of the baseline value. FEV₁ was measured every 15 min until the EAR had resolved. FEV₁ was then measured every hour until a total of 12 h had elapsed after allergen challenge. LAR was diagnosed when a second drop in FEV₁ $\geq 15\%$ of the baseline value was obtained.

CIE and CRIE

A serum sample of each patient was obtained the day of the BPT for the determination of specific IgE for cat by the RAST technique (Phadezym RAST®, Pharmacia) and for CRIE analysis.

Before CRIE, a CIE was performed. The first dimension of the CIE was obtained by migration of 30 μ g of the cat extract (Pharmalgen®, Pharmacia) on agar gel (Agarose Litex HSA with Tris-Barbital Buffer, pH 8.6) at 10 V/cm for 40 min. The second dimension was obtained by migration of the separated proteins of the cat extract on agar gel containing 125 μ l of rabbit anti-cat IgG (generous gift from R. Einarsen, Pharmacia, Sweden) at 2 V/cm for 22 h. The gel was then stained by Coomassie Brilliant Blue for 5 min (Fig. 1). Throughout the whole study, the same lot of cat extract was used. The extract inhaled during the BPTs was the same that we used for the CIE/CRIE technique in our 21 patients.

The CRIE was performed by incubation of the unstained CIE with each patient's serum diluted 1/15 in phosphate buffer (pH 7.0) for 20 h. The gel was incubated with ¹²⁵I-labelled anti-IgE (Pharmacia) diluted 1/15 in phosphate and Tween 1% buffer at 180 000 c.p.m. for 20 h. The gel was dried and exposed for autoradiography to a Kodak XL-1 film within a Kodak X-Omatic cassette containing an intensifying screen for 10 days at -80°C . CRIE were also performed with sera from three non-atopics and three patients allergic to grass pollen

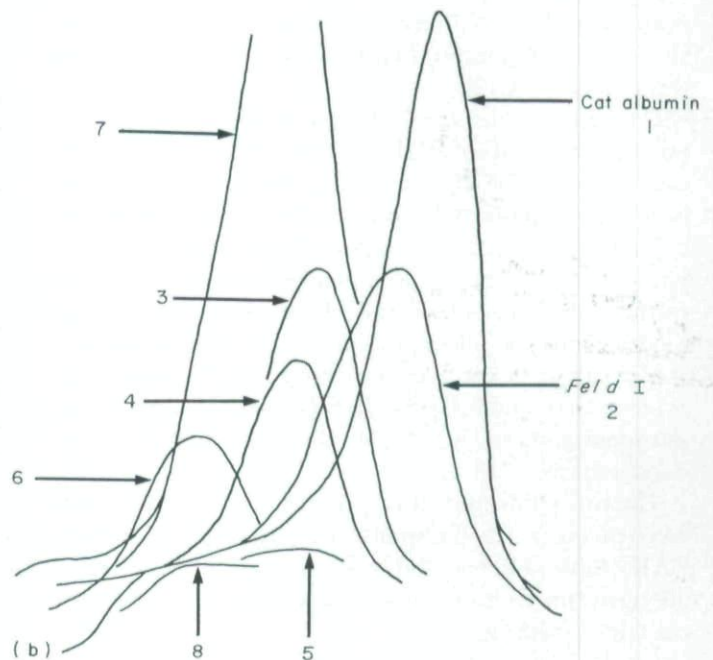
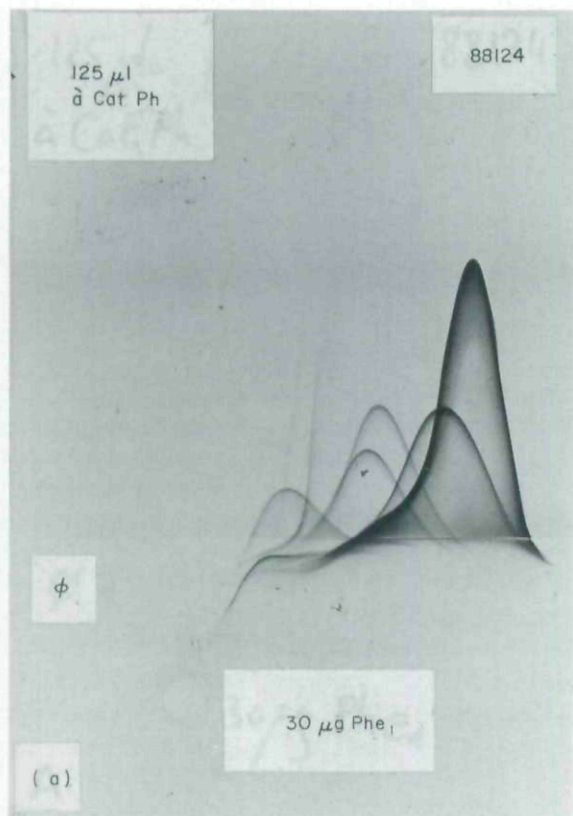


Fig. 1. a) CIE of the cat extract. b) Corresponding antigens.

(RAST ≥ 17.5 PRU/ml, Pharmalgen® G $\times 3$), with a negative RAST to cat. A semi-quantitative score of radiostaining was performed using the Mancini technique [21]: ^{125}I -anti-IgE was diluted to nine different concentrations with rabbit IgG, and the mixture allowed to diffuse in an agar gel containing swine anti rabbit IgG. Autoradiography of the gels was performed as above, and the intensity of the radiostaining scored from 0 (no band) to 9 (intense band). All incubations were made at room temperature.

Statistical analysis

Differences between patients with LAR and patients without LAR in radiostaining score, number of antigens on CRIE, baseline FEV₁, FEV₁ drop in EAR, and dose of inhaled allergen were analysed by the Mann-Whitney test; $P < 0.05$ was considered significant. RAST results (expressed in PRU/ml) were analysed with confidence intervals for differences between means. Prevalence of specific IgE to cat albumin, *Fel d I* and protein #7 on CRIE was analysed with the chi-square test. Confidence limits of the sensitivity and specificity of protein #7 for LAR were determined with a table of confidence limits for small numbers [22].

Results

BPT results

All 21 patients reacted to the BPT with an EAR. The drop in FEV₁ ranged from 20% to 55% ($34 \pm 10\%$; mean $\pm$ s.d.) One hour after the last allergen inhalation, FEV₁ in all 21 patients was $\geq 94\%$ of the baseline value. Eight of the 21 patients had a LAR with a second drop in FEV₁ ranging from 15% to 49% ($32 \pm 4.3\%$). No significant difference was found in the recovery pattern from the EAR between patients who did and patients who did not develop a LAR. The comparisons of baseline FEV₁, FEV₁ drop in EAR, and cumulated dose of inhaled allergen for the 13 patients with EAR only and the eight patients with EAR + LAR are presented in Table 1. The baseline FEV₁ and the cumulative dose of inhaled allergen were not significantly different between the two groups of patients. The subjects with EAR + LAR had a deeper drop in FEV₁ than the 13 subjects with EAR only, but this difference was not statistically significant. Patients with EAR + LAR had a higher mean level of specific anti-cat IgE, than patients with EAR only (51.3 PRU/ml and 14.5 PRU/ml respectively). The difference between medians of the two groups was -15.9 with a 95% confidence interval ranging from -46.1 to -2.4 .

Table 1. Comparison of baseline FEV₁, FEV₁ drop in EAR and amount of allergen inhaled between patients with EAR only and patients with LAR (mean $\pm$ SD)

	Patients with EAR only	Patients with EAR + LAR	P*
Baseline FEV ₁ (% of predicted)	91% ($\pm$ 8%)	92% ($\pm$ 9%)	n.s.
FEV ₁ drop in EAR	29.3% ($\pm$ 6.3%)	41.7% ($\pm$ 10%)	n.s.
Inhaled allergen (BU)	32.5 $\times$ 10 ³ ($\pm$ 5.3 $\times$ 10 ³)	12.4 $\times$ 10 ³ ($\pm$ 14.8 $\times$ 10 ³)	n.s.

* Mann-Whitney; n.s., not significant.

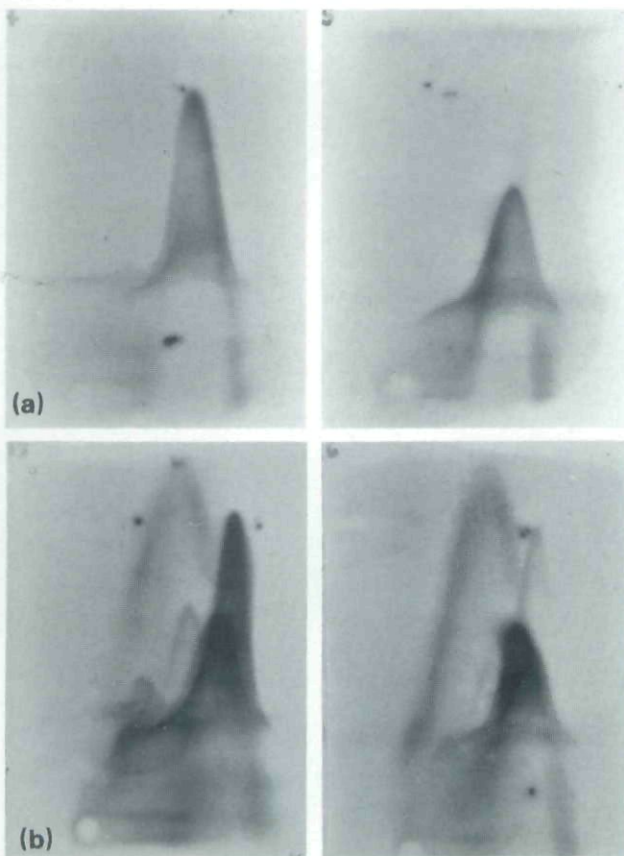


Fig. 2. CRIE pattern of two patients with EAR and two patients with EAR + LAR. a) Patients with EAR only; IgE binding to *Fel d I* and cat albumin; b) patients with EAR + LAR; IgE binding to Ag no. 7.

CRIE results

As shown in Fig. 1, the cat extract we used was similar but not identical to that published by other investigators [15–17].

Figure 2 shows the CRIE of two patients with EAR

only and two patients with EAR + LAR. The number of the cat extract proteins that bound to IgE varied from 1 to 5 (mean: 2.8 ± 1.2) in patients without LAR and from 3 to 6 (mean: 3.9 ± 1) in patients with LAR (Table 2). IgE from both groups of patients detected cat albumin and *Fel d I*. However protein no. 7 bound to IgE of all eight patients (100%) with LAR, but only five of the 13 patients (38%) without LAR (Table 2). Protein no. 7 sensitivity for LAR was 100% (95% confidence limits; 63.1–100%) and specificity was 61.5% (95% confidence limits; 31.6–86.1%). The other five proteins present in the cat extract bound less frequently to IgE and no differences between the two groups could be detected. The control CRIE performed with sera obtained from non-atopic patients or from atopics with negative RAST to cat all showed blank films on the autoradiograms (Fig. 3).

The total score of radiostaining was higher for patients with LAR than for patients without LAR (Table 2). The scores of radiostaining of the 21 patients for cat albumin, *Fel d I* and protein no. 7 are presented in Fig. 4. The eight patients with LAR all had a score of at least 5 on each of these three proteins.

Discussion

Since LAR may be a better model of natural clinical asthma than EAR, it is important to detect and study patients who may develop such reactions. Hargreave *et al.* have shown that patients who have a deep drop of FEV₁ in EAR are more likely to develop LAR [23]. Our study confirms this finding since our patients with LAR had a mean FEV₁ drop of 42% in EAR compared with 29% in patients who did not develop a late reaction. However, this difference did not reach statistical significance. Moreover, the recovery from EAR was not different in the two groups of patients, and FEV₁ was nearly back to baseline in all subjects 1 h after the last dose of allergen. BPT may detect patients at risk of developing severe

Table 2. Cat extract's antigens present on CRIE

	Patients with EAR only	Patients with EAR $\pm$ LAR	<i>P</i>
Total score (mean $\pm$ SD)	15 $\pm$ 7 (range: 4–28)	24 $\pm$ 5 (range: 20–35)	n.s.*
Number of antigens	2.6 $\pm$ 1.2 (range: 1–5)	3.9 $\pm$ 1 (range: 3–6)	n.s.*
Cat albumin	Present in 12/13 patients	Present in 8/8 patients	n.s.**
<i>Fel d</i> I	Present in 12/13 patients	Present in 8/8 patients	n.s.**
Protein no. 7	Present in 5/13 patients	Present in 8/8 patients	<0.005**

*, Mann-Whitney; **, Chi-square; n.s., not significant.

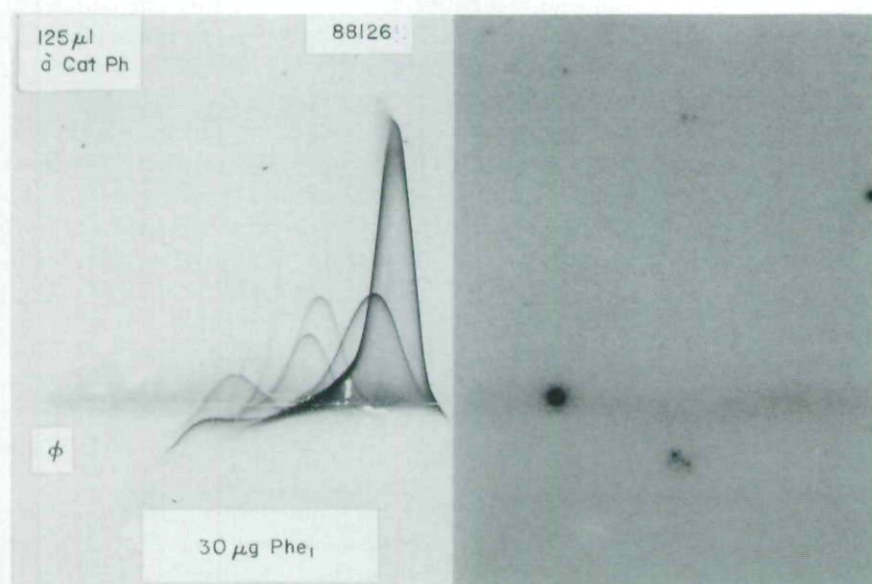


Fig. 3. CRIE (right) performed with the serum of a patient allergic to grass pollen (RAST > 17.5 PRU/ml), but not allergic to cat (negative RAST). No IgE binding to the cat extract components could be detected after 10 days of autoradiography. The CIE of the cat extract used for this experiment is presented at left.

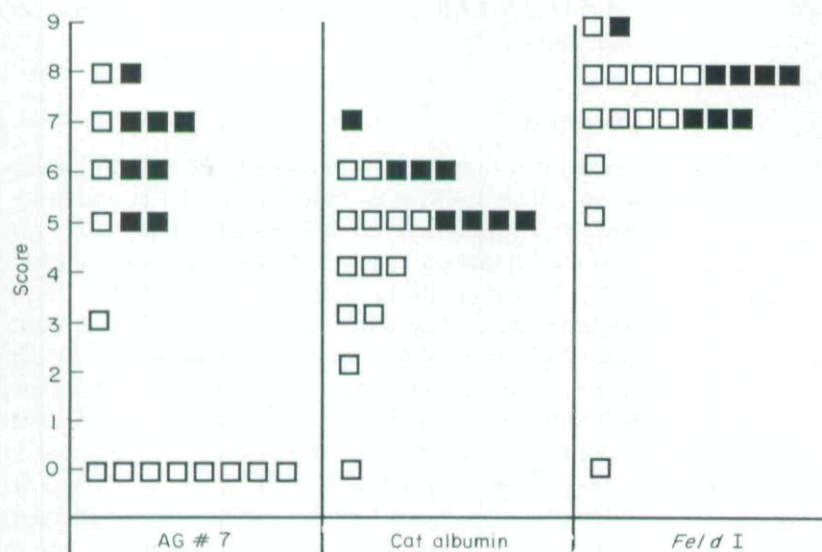


Fig. 4. Scores of the 21 patients for antigen no 7, cat albumin and *Fel d* I. □. Patients with EAR only. ■. Patients with EAR + LAR.

asthma but must be reserved for special and well defined indications [1,2]. Although small doses of allergen can elicit a LAR [24], LARs are more common after high doses of allergen [25,26]. In our study, the dose of inhaled allergen was not predictive of the LAR. Indeed, we were unable to correlate its occurrence with the amount of inhaled allergen. Some authors have proposed methods and indexes to predict who will develop LAR [27–29], and LARs have been shown to enhance bronchial responsiveness [30]. High levels of specific IgE are correlated with the occurrence of LAR [24]. Only three of our 13 patients with EAR had a RAST class of 4 compared with 6/8 patients with EAR + LAR. When the specific IgE levels were expressed in Phadezym RAST Units/ml (PRU), this trend was even more obvious. This observation confirms that a high level of sensitization plays a key role in the occurrence of LAR. However, while the RAST detects IgE directed against an allergen extract as a whole, CRIE allows more precise detection of which proteins in the allergen extract bind to specific IgE. The analysis of the CRIE patterns of our patients suggests a correlation between the CRIE results and the incidence of LAR to cat after BPT. Patients with LAR had a greater IgE response to the extract. The CRIE radiostaining score was higher, and more components of the cat extract were bound by IgE of patients with LAR than patients without LAR. Twenty of 21 patients had specific IgE for *Fel d I* which is the major allergen of cat extracts. We also found 20 of 21 patients with specific IgE for cat albumin, which is not in agreement with some previous reports [31]. This high incidence of specific IgE for cat albumin in our study may be due to a higher concentration of cat albumin in our cat extract. Moreover, it has been shown that specific IgE to cat albumin may sometimes be detected on the autoradiograms only after several days [15]. Protein no. 7 bound the IgE of 100% of patients with LAR but only 38% of patients who did not develop LAR. Only patients with LAR had high radiostaining scores with all three proteins no. 1 (cat albumin), no. 2 (*Fel d I*) and no. 7. Non-specific binding of IgE to proteins of the cat extract is unlikely, since CRIE performed with sera from atopic patients with negative RAST to cat showed no binding.

These findings indicate a correlation between the IgE-CRIE pattern and the occurrence of late allergic reactions to cat. Moreover, the presence of specific IgE to protein no. 7 might be a more powerful trigger of the allergic reaction than the presence of IgE to other components of the cat extract. As the concentration of allergens may vary in cat extracts from different sources, it would be worth repeating these experiments with other cat extracts.

Further studies are needed to explain the possible role of protein no. 7 in the pathogenesis of LAR after BPT with cat extracts. These include characterization of

antigen no. 7 in more detail, determination of its source and airborne characteristics, BPT and skin testing with purified proteins rather than whole cat extracts, and measures of baseline airway responsiveness to metacholine or histamine (which were not conducted in this study), to determine if there is a link between the degree of bronchial hyperresponsiveness and the CRIE pattern of allergic asthmatic patients.

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